

[COMMUNICATION]

Transient Increase in Mitochondrial Protein Synthesis of Starfish Embryo before Gastrulation

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ABSTRACT—A transient increase in protein synthesis *in vitro* was observed in mitochondrial fraction of starfish embryos at early gastrula stage. This stimulated activity was inhibited by chloramphenicol but not by cycloheximide. The purification of the protein synthesized in mitochondrial fraction was performed by column chromatography after labeling with [³H]-amino acids and the peak fraction of radioactivity was analyzed by SDS-polyacrylamide gel electrophoresis. The result shows that there was only three species of newly synthesized proteins in mitochondrial fraction of starfish embryos. The molecular weights of these proteins were estimated about 62, 54 and 47 kD respectively.

INTRODUCTION

The mechanism of differentiation at gastrulation of sea urchin embryos has not been elucidated yet. In a first step of this research, the effect of lithium chloride or other heavy metals had been examined. When embryos were treated with lithium chloride, invagination of archenteron at mesenchyme blastula stage was inhibited and the archenteron was turned inside out [1-4]. This type of gastrula embryos is called exogastrula. Some kinds of antibiotics were also responsible for the suppression of gastrulation. Chloramphenicol which is one of effective antibiotics for the induction of exogastrula [5-7] inhibits protein synthesis in prokaryote and mitochondria through the reaction with 70S ribosomes. In the previous paper, the relationship between the gastrulation of sea urchin embryo and mitochondrial protein synthesis was

examined. The protein synthetic activity *in vitro* was stimulated transiently at the mesenchyme blastula stage by the activation of peptide elongation factor in mitochondria [8]. In this paper, we examined characteristics of synthetic proteins in mitochondrial fraction of starfish embryos.

MATERIALS AND METHODS

Starfish embryos

Gonads were removed from the arms of mature starfish (*Asterina pectinifera*). The testes were placed on Petri dish on ice until used. The ovaries were removed and placed in a dish containing calcium-free sea water. After 30 min, the follicle-free ovaries were collected with nylon mesh, and washed twice with artificial sea water. Then the oocytes spawned by the addition of KCl were treated with 10⁻⁶ M 1-methyladenine. Over 95% of the oocytes showed germinal vesicle breakdown at 30 min after the treatment with 1-methyladenine. Then, the diluted sperm were added and fertilized eggs were cultured in filtered sea water at 18±2°C with gentle agitation.

Preparation of mitochondrial protein and column chromatography

The preparation of mitochondrial fraction and measurement of [³H]-amino acids incorporation were performed as the previous paper in sea urchin embryos [8].

The column chromatography was carried out at 4°C. After the treatment of [³H]-amino acids,

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mitochondrial fraction was solubilized by the addition of 0.1 vol of 10% Triton X-100. The solution was filtered by TOYO-ultrafilter UP-20, size 43. Then the membrane was washed with buffer A consisted of 0.1 M NaCl and 50 mM Tris-HCl (pH 8.0) and the protein fraction obtained was concentrated to the volume of 1 ml. Thus the mitochondrial protein from 1 ml of packed cells was suspended in 1 ml of buffer A. The suspension was applied on a Sephadex G-100 column (15×300 mm) which was equilibrated and eluted with buffer A at a constant flow rate (8 ml per hour). Each 2 ml of the eluate was collected by a fraction collector. The 500 μ l of each fraction was added to 5 ml of AQUASOL-2 and the radioactivity was counted with a liquid scintillation counter (ALOKA LSC-700). Fractions at the peak of radioactivity were collected and concentrated by ultrafiltration. The sample was diluted with the same buffer and applied on a DEAE Sephadex A-50 column (15×300 mm). The elution was carried out by a linear gradient concentration of 0.1 M to 1.0 M NaCl in the buffer. The flow rate was 8 ml per hr and each 2 ml of fraction was collected. The incorporated radioactivity was counted.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli [9], using 12.5% polyacrylamide gel.

Protein measurement was carried out according to the method of Lowry *et al.* [10], using the bovine serum albumin as standard.

RESULTS AND DISCUSSION

Protein synthetic activity in mitochondrial fraction of starfish embryo

Figure 1 shows *in vitro* incorporation of [³H]-amino acids into the mitochondrial protein of developing starfish embryos in the presence or absence of chloramphenicol and cycloheximide. Protein synthetic activity in mitochondrial fraction was increased at the late blastula stage. The peak was observed at the early gastrula stage and the activity decreased to the low original level at the

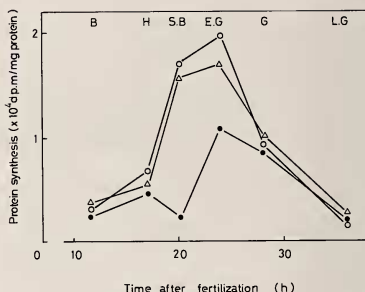


FIG. 1. Changes in [³H]-amino acids incorporating activity into mitochondrial protein of starfish embryos during development. Reaction medium and procedures are described in text. Other additions are indicated as none (○), 100 μ M cycloheximide (△) and 100 μ M chloramphenicol (●). Characters in the uppermost part of the figure indicate stages of development: B, blastula; H, hatching; EG, early gastrula; G, gastrula and LG, late gastrula.

gastrula stage. The addition of 100 μ M chloramphenicol suppressed the protein synthesis between late blastula and early gastrula stage. In the case of sea urchin embryo, the addition of 35.5 μ M cycloheximide did not inhibit the protein synthesis in the mitochondria but rather stimulated [8]. In starfish embryos, the protein synthesis was not stimulated by cycloheximide. It seems that the stimulation of the protein synthesis in mitochondrial fraction may be regulated by different fashions between these two species.

Purification of the protein synthesized in mitochondrial fraction

To purify the protein synthesized in mitochondrial fraction, the mitochondrial fraction was incubated with [³H]-amino acids, solubilized by Triton X-100, and applied on Sephadex G-100 and DEAE Sephadex A-50 columns. The elution pattern of the radiolabeled proteins by Sephadex G-100 column is shown in Figure 2. Although there was only one peak of the synthesized protein of mitochondrial fraction in the elution pattern of Sephadex G-100 column, several components were

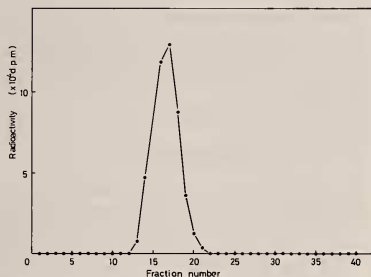


Fig. 2. Sephadex G-100 column chromatography. The mitochondrial fraction of starfish embryos was solubilized by Triton X-100 and loaded onto Sephadex G-100 column (15×300 mm). The column was eluted with buffer A (see text). Closed circle shows the elution pattern of the newly synthesized protein monitored by incorporated radioactivity.

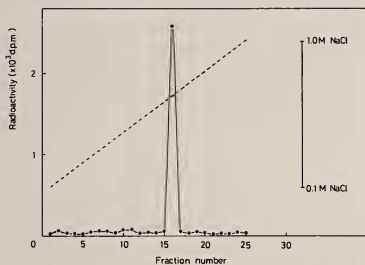


Fig. 3. DEAE Sephadex A-50 column chromatography. The peak fraction obtained from Sephadex G-100 was concentrated by ultrafiltration and applied on DEAE Sephadex A-50 column. The column was eluted with 0.1 to 1.0 M NaCl gradient. Closed circle shows the newly synthesized protein monitored by incorporated radioactivity (solid line). Broken line indicates NaCl concentrations.

recognized by SDS-polyacrylamide gel electrophoresis (Fig. 4-C). Then the Sephadex G-100 peak fractions were concentrated by ultrafiltration and eluted through a column of DEAE Sephadex A-50. As seen in Figure 3, the protein synthesized in mitochondrial fraction was eluted from the column at about 0.75 M NaCl. The peak of radioactivity was observed in only one fraction.

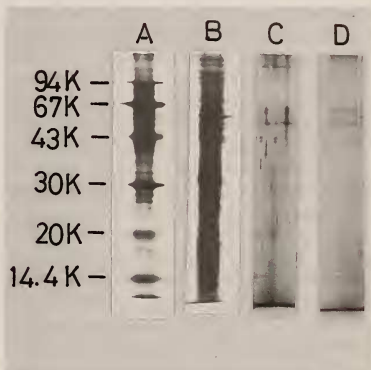


Fig. 4. Polyacrylamide gel electrophoresis of proteins from mitochondrial fraction at early gastrula stage of starfish embryos. Alphabet at the top of figure indicate as follows; A: low molecular weight standard, B: whole mitochondrial protein at early gastrula stage of starfish embryos, C: peak fraction of Sephadex G-100 column, D: peak fraction of DEAE Sephadex A-50 column.

Analysis of the purified protein by SDS-PAGE

The mitochondrial protein labeled with [3 H]-amino acids were examined by SDS-PAGE after the purification by two types of column chromatography. Figure 4 shows the results of SDS-PAGE using 12.5% polyacrylamide gel. When proteins from whole mitochondrial fraction at mesenchyme blastula stage was analyzed, many bands were observed (Fig. 4-B). In the peak fraction obtained from Sephadex G-100 column chromatography, three main and several minor bands were found in the high molecular weight region in SDS-PAGE (Fig. 4-C). After the DEAE Sephadex A-50 column chromatography, three major bands in SDS-PAGE were recognized in the peak fraction (Fig. 4-D). The molecular weight of these proteins were estimated as about 62, 54 and 47 kD, respectively. It has been known that the molecular weight of enzyme proteins located in mitochondria is in general 7.5 kD to 45 kD. Since all of proteins synthesized in mitochondria of starfish embryos had higher molecular weights

than that in general mitochondrial proteins, synthesized proteins might not be respiratory enzymes which have lower molecular weight. If the phenomenon observed in this paper were concerned with the multiplication of mitochondria itself, the synthesized proteins would be a constitutive protein of mitochondria

There are several recent indications for specific mitochondrial changes in morphology and functions during early development of animal embryos. In our previous paper, a transient increase in protein synthesis in mitochondria was observed in mesenchyme blastula of the sea urchin. In the mouse embryo, mitochondrial protein synthesis *in vitro* was also indicated during oogenesis and early embryogenesis [11, 12]. Furthermore, mitochondria might play an important role in the embryonic development of insects. It has been indicated that cytoplasmic factor in the polar region of insect embryos is encoded by mitochondrial genome. Mitochondria in the posterior pole of *Drosophila* eggs have been recognized to attach to polar granules which differentiate to primordial germ cell during development. As well as mitochondrial protein synthesis in starfish embryo, some sort of essential informations for embryonic differentiation might be stored or formed in mitochondria at

a certain stage of development in other kinds of embryo. The relationship between the transient increase of protein synthesis in mitochondria and differentiation of embryos should be analyzed furthermore.

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